# EXPERIMENTAL MIXING IN A NORTH TEMPERATE LAKE: EXAMINATION OF VARIABILITY IN SPATIAL AUTOCORRELATION IN FISH AND ZOOPLANKTON POPULATIONS

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## Abstract

In lakes, biotic and abiotic variables interact at multiple spatial and temporal scales, resulting in heterogeneous horizontal distributions of organisms. Although habitat heterogeneity is a vital aspect of ecosystem function and performance, few studies recognize spatial autocorrelation and scale dependence of biotic communities within their abiotic environment. Zooplankton and zooplanktivorous fish represent two trophic levels of pelagic lake food webs whose heterogeneous horizontal distributions may be affected by water movements, prey availability, predatory avoidance, swimming capacity, and thermal tolerance. The most active driver of these distributions depends heavily on scale of analysis. We used hydroacoustic surveys and variogram analysis in a small, north-temperate lake in Vilas County, WI to compare whole-lake horizontal patterns of fish and zooplankton separately, then examined patterns in their interactions. We tested the durability of these patterns in response to a whole-lake manipulation project in which we experimentally destratified the lake, and examined how horizontal patterns change in response to alteration in vertical structure. Lake manipulation decreased the scale of spatial autocorrelation for fish populations. Fish subsequently returned to pre-manipulation spatial patterns after mixing ceased, suggesting the manipulation induced a flight response by fish in response to removal of preferred coldwater habitat. We did not detect changes in zooplankton spatial patterning with mixing, but found support for the hypothesis that external factors, such as wind and wind-induced water movements, may drive large scale horizontal spatial patterns in zooplankton distributions. We also found that fish and zooplankton aggregations exhibit spatial structure at different scales, which may have implications for sampling designs measuring both fish and zooplankton. We detected bottom-up effects where fish aggregate in regions of high zooplankton biomass for at least a portion of the year, and did not detect top-down effects.

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## Introduction

Identifying biotic and abiotic processes that result in coupling behavior of multiple trophic levels, where the distribution of one level affects the distribution of another, is a vital component of understanding lake ecosystem structure and function (Pinel-Alloul 1995, White and Brown 2005). These processes may be interwoven, and create a heterogeneous environment where distributions of fish and zooplankton are patchy and unevenly distributed throughout the system (Turner et al. 2001, Rinke et al. 2009). Processes that structure spatial heterogeneity in lakes include light intensity, dissolved oxygen, weather-induced water movements, food availability, predator avoidance, behavioral movements, and thermal stratification (Pinel-Alloul 1995, Folt and Burns 1999).

Spatial heterogeneity is vital to ecosystem performance, as it facilitates multi-species coexistence by providing a range of niches for species to occupy (Hanski 1981, Ross 1986). For example, thermal stratification may allow multiple species to inhabit areas based on temperature-dependent metabolisms (Brown et al. 2004). Stratification also provides thermal refuge from predation (Tessier and Welser 1991, Pangle and Peacor 2006).

Fish and zooplankton represent two trophic levels of pelagic food webs whose heterogeneous distributions are affected by water movements, prey availability, predatory avoidance, swimming capacity, and thermal tolerance (Rose and Legget, 1990, Folt and Burns 1999, Hrabik et al. 2006, Jensen et al. 2006, Blucakz 2009, Holbrook 2011).

However, few studies have simultaneously examined fish, zooplankton, and abiotic processes that may affect horizontal spatial distributions.

Single abiotic and biotic processes usually do not describe pattern formation in distributions. More likely, there is a multifactorial suite of variables acting simultaneously to determine aggregations, as described in the "Multiple Driving Forces Hypothesis" (Pinel-Alloul 1995). For example, wind-induced water movements may cause down-wind accumulation of zooplankton, but wind does not fully explain the vertical change in zooplankton density, which is more likely caused by biotic processes such as food availability and predatory avoidance (Pinel-Alloul 1995, Rink et el. 2009, Holbrook 2011). Further, distributions may be species and size-specific, and are likely to vary across systems (George and Winfield 2000, Ahrenstroff et al. 2013).

The intensity of drivers within the multiple driving relationships depends heavily on the spatial and temporal scales of measurements (Pinel-Alloul 1995, Rinke 2009). At a small scale (submeter-1,000m) individual predatory-prey encounters are highly influential on distributions, causing fish and zooplankton assemblages to overlap (Malone and McQueen 1983, Pinel-Alloul 1999). At a large scale (> 1km in past studies conducted in large water bodies) the effect of predator-prey encounters is less influential, and assemblages may not overlap but may still be located in close proximity as a result of thermoregulation and bioenergetic efficiency (Rose and Leggett 1990, Rinke 2009, Holbrook 2011). Past studies have examined drivers of both zooplankton and fish horizontal distributions independently, but few have concurrently studied interactions between fish, zooplankton, and physical processes. Understanding each group

individually is important, but examining the interplay of all groups simultaneously is essential to gain an ultimate understanding of ecosystem function in terms of spatial extent of interactions across trophic levels.

*Factors affecting zooplankton horizontal distributions*

Zooplankton distributions are affected by trade-offs between predatory avoidance and prey availability, as well as physical processes such as temperature, light intensity, and water movements caused by weather events (Folt and Burns 1999, George and Winfield 2000). At a small scale (submeter-1,000m), distributions are likely to be affected by biotic factors such as predator avoidance, food availability, and swarming behavior (Davies 1985, Jakobsen and Johnsen 1988, Dodson 1990). However at large spatial scales, studies have found persuasive evidence for the effects physical process such as currents, eddies, wind, and internal waves, especially when lakes are oriented parallel to the prevailing wind direction (Thackeray et al. 2004, Rinke et al. 2007, Blukacz et al. 2009, Rink et al. 2009, Pernica et al. 2013). These relationships are related to the "conveyor belt hypothesis," in which wind-driven water results in large, downwind accumulations of zooplankton (Ragotzkie and Bryson 1953). Other wind-driven processes, such as Langmuir circulation cells, are highly influential on distributions of zooplankton (Wetzel 2001, Pernica et al. 2013).

*Factors affecting fish horizontal distributions*

Planktivorous fish distributions may be affected by patchy zooplankton distributions, which act as bottom-up drivers when fish are feeding by affecting growth, production,

and survival (Ware and Thomson 2005). Planktivorous fish may also be driven by tradeoffs to maximize predation on zooplankton, while also minimizing predation risk to piscivores (Hrabik et al. 2006, Jensen et al. 2006). Positive relationships, where zooplankton act as a driver of fish distributions, have been found in several studies examining both pelagic and littoral communities (Rinke 2009, Holbrook 2011, Ahrenstorff et al. 2013). At a large, pelagic spatial scale (200-800m in 64ha-15,000ha inland lakes) Holbrook (2011) found consistent bottom-up effects of zooplankton driving fish distributions for inland lakes, and inconsistent effects of physical processes, such as wind-driven water movements, on fish distributions in small and large lakes. This may suggest abiotic drivers on fish distributions may be system-dependent, varying by lake size, bathymetry, and habitat structure. Future research may require a combination of both large and small scale analyses across several systems (Romare et al. 2003, Ahrenstorff et al. 2013).

*Interactions between fish and zooplankton*

Few studies have concurrently examined the interactions between fish, zooplankton, and abiotic processes that affect horizontal distributions at a large, whole-lake spatial scale. Further, studies attempting to examine these patterns have been conducted in large water bodies, and current research lacks examination of spatial patterning in small lakes (George and Winfield 2000, Thackeray et al. 2004, Blukacz et al. 2009, Rinke et al. 2009). Although drivers of fish may be system or species dependent, there is a more consistent bottom-up effect of zooplankton on fish distributions (Romare et al. 2003, Holbrook 2011, Ahrenstorff et al. 2013). It is possible

that large-scale studies are not able to detect species-specific interactions that could sometimes act as top-down drivers, where zooplankton aggregate in certain areas of the lake to avoid predation or when fish select for specific sizes or species (Brooks and Dodson 1965). Zooplankton are more likely to be affected by individual predator-prey encounters with fish and other zooplankton at smaller scales, but many studies demonstrate physical processes such as wind, currents, and upwellings are the main driver of zooplankton distributions at a large spatial scale (Folt and Burns 1999, Blukacz et al. 2009, Holbrook 2011).

Management of fish and zooplankton communities requires knowledge of their interactions within the context of their physical and biological settings in order to identify scale of activity (foraging, predator avoidance, migration, etc.), gain understanding of factors affecting fish and zooplankton across spatial scales, and therefore predict limits and extent of management decisions across distances (Durance et al. 2006). Although habitat heterogeneity is a vital aspect of ecosystem function, few studies recognize spatial autocorrelation of biotic communities within their abiotic environment. Further, patterns affecting fish and zooplankton distributions may occur at different spatial scales and result from different processes, stressing the need to examine populations across increasing spatial and temporal scales (Durance et al. 2006).

Of the abiotic factors that structure spatial heterogeneity, thermal stratification is one of the most central in small freshwater lake ecosystems. Thermal stratification in the pelagic zone of freshwater lakes structures heterogeneous physical habitat, and has far-reaching effects, including determination of the rate of chemical and biological processes,

influence on phenology of aquatic animals, species distribution, and predator-prey interactions (Sommer et al. 1986, Tessier et al. 1991, MacPhee et al. 2011, Tessier at al. 2011). Furthermore, spring and fall turnover replenish oxygen and nutrients throughout the lake. Annual progression of thermal gradients also determines limnological sampling design, and as a result, a great deal of limnological research relies on assumptions that mixing and stratification are central to cyclical biological events (Sommer et al. 1986, Wetzel 2001). Central theories developed through reliance on stratification components often serve as foundations for additional theories and concepts. There is therefore a need for an experimental elimination of stratification to fully assess the role of thermal stratification in regulation of trophic dynamics. A full manipulation of thermal stratification would aid in accurately assessing freshwater population responses to future disturbance events such as climate change, major precipitation events, and water reservoir releases that may alter vertically structured processes. Manipulation would also aid in determination of ultimate causes of spatial distributions of multiple trophic levels at a large scale.

A cross-disciplinary team of physical, biological and chemical scientists completed a whole-lake manipulation of Crystal Lake, WI resulting in complete destratification of thermal gradients in two summer seasons (Lawson et al. 2015). This experiment provided an opportunity to examine potential changes in horizontal spatial distributions of fish and zooplankton in response to mixing and loss of vertical spatial heterogeneity. By comparing pre-manipulation and manipulation data, the effects of thermal structure on distributions and movements of fish and zooplankton can be

determined. Additionally, monitoring the lake post-manipulation will yield insight as to how quickly fish and zooplankton distributions are able to return to a preexisting stratified condition. Hydroacoustic technology is ideal for these analyses, as it is able to quickly and simultaneously sample fish and zooplankton communities over large areas, and it provides an opportunity to examine horizontal spatial relationships between fish and zooplankton at a fine spatial scale. In this study, we used hydroacoustic data and semivariogram analyses to examine horizontal spatial relationships between fish and zooplankton, before (2010-2011), during (2012-2013), and after (2014) mixing. This research will aid in determining the role of stratification in food web interactions, yield information regarding physical and biological coupling, and indicate lake responses to a disturbance regime (induced mixing).

We examined horizontal spatial relationships of fish and zooplankton separately, and then examined the spatial relationship between fish and zooplankton. We hypothesized that zooplankton aggregations would be smaller and more discrete (small range values (m) in semivariogram analysis) pre-mixing as a response to biotic events such as food availability, predatory avoidance, and swarming behavior (Davies 1985, Jakobsen and Johnsen 1988, Dodson 1990). We predicted aggregations would become larger (increase in range values (m) in semivariogram analysis) with mixing due to redistribution in resources (potential homogenization of preferred habitat and food resources), and that distributions would return to pre-mixed patterns once mixing ended. In addition, we hypothesized zooplanktivorous fish would exhibit a positive, bottom up

response to zooplankton aggregations, resulting in similar changes in horizontal distributions (Rinke et al. 2009, Holbrook 2011).

## Materials and Methods

### *Crystal Lake*

Crystal Lake is a small, 34 hectare oligotrophic seepage lake located in Vilas County, WI. The lake has a maximum depth of 20 meters, average Secchi depth during the ice-free period of 8 meters, and a well-oxygenated (6-7 mg/l) hypolimnion that has a mean temperature of 5-7° C during the summer. The littoral habitat is sandy with little coarse woody debris, and few bryophytes. Crystal Lake is also a North Temperate Lakes Long Term Ecological Research (NTL-LTER) lake, and has over 30 years of abiotic and biotic limnological data available.

Historically, Crystal Lake was dominated by yellow perch (*Perca flavescens*) until the introduction of rainbow smelt (*Osmerus mordax*) in 1985. Post introduction, rainbow smelt became the dominant species in Crystal Lake. Currently, smelt make up about 99% of the population in Crystal Lake, while yellow perch make up the majority of the remaining 1%.

### *Thermal Destratification in Crystal Lake*

In 2012 and 2013, thermal manipulation in Crystal Lake began post ice-out before the onset of late spring stratification. Gradual Entrainment Lake Inverters (GELIs) were used to continuously mix the lake, increase heat flux into the lake, and prevent stratification. GELIs are large, 8-meter diameter trampoline-like discs consisting of a

membrane bound to a stainless steel frame. A central air bladder is located in the middle of the GELIs, which is able to inflate and deflate via control by a central computer station that relayed commands to an off-shore pump house containing several air compressors. Air was sent through underwater tubing to the GELIs. Six GELIs were located in a circular pattern in the deeper basin of Crystal Lake (Figure 1). Constant cycling of GELIs created a large amount of surface drag throughout the water column. By mixing cold water from the bottom with warmer top waters, GELIs formed a homogenous temperature throughout the entire water column (Lawson et al. 2015).

*Hydroacoustic Data*

Hydroacoustic surveys were conducted on Crystal Lake every other week from late May to early November during years of pre-manipulation (2010 and 2011), manipulation (2012 and 2013), and one year post-manipulation (2014). Data were collected in pelagic regions (>8m depth) at night (at least one hour after sunset) to ensure fish were dispersed in the water column. Schooling, which commonly occurs during the day, induces error in hydroacoustic estimates (Appenzeller and Legget 1991, Parker-Setter et al. 2009). We used a Biosonics DTX echosounder with 123- and 430-kHz split-beam transducers mounted to a tow body (Biofin, Biosoincs Inc.) and towed next to the boat at 0.5 m depth with a vessel speed not exceeding 3mph (2.6 knots). The 123-kHz transducer was used to measure backscatter from fish targets with low interference from zooplankton, and the higher frequency 430-kHz transducer was used to measure backscatter from zooplankton within the water column.

We verified the accuracy of transducer calibration during every sampling event by using standard tungsten-carbide spheres to ensure the received target strengths did not vary from expected target strengths for each sphere (<±1 dB) on any date. A GPS was used concurrently with hydroacoustic sampling to obtain latitude and longitude. Acoustic transects on Crystal Lake in 2010, 2011, and 2012 were conducted in a zig-zag pattern. Transects during 2013 and 2014 were composed of 6 parallel lines running north-south (Figure 1). We used a pulse duration of 0.4 milliseconds at 3 pulses per second, and a minimum threshold for raw echo inclusion of -70dB for 123kHz and -100dB for 430kHz using 20logR echo integration. Crystal Lake was equipped with a profiling buoy to obtain average water column temperatures, which we used to calculate the sound speed through water. Hydroacoustic data were analyzed using Echoview software (v.4.1).

*Fish densities*

We removed bottom echoes from each sample date by excluding 0.5 m from the bottom. The bulk of the fish and zooplankton biomass was located below the thermocline and we sampled only pelagic waters so we performed no vertical binning of the samples. Data were analyzed in 50m horizontal bins. For each horizontal bin we calculated areal density ($\rho_{as}$) of number of fish per $m^2$. We used a proportion of number of specimens in each species ($p_s$ ) of 1 because we were interested in all fish rather than specific species, although we expect almost all fish to be rainbow smelt because smelt accounted for 99% of pelagic gill net catches, with the remaining portion being yellow perch.

We first calculated the weighted mean backscattering cross-section:

$$\overline{\sigma_{bs}} = \sum (p_s * 10^{\frac{TS_s}{10}})$$

where *TS* is the target strength from an individual specimen. We then calculated volumetric density:

$$\rho_{vs} = \frac{p_s}{\overline{\sigma_{bs}}} * s_v$$

where $s_v$ is the linear mean volume backscattering strength for each cell, which is calculated from the echo integration file of the echogram. We finally calculated fish areal density:

$$\rho_{as} = \rho_{vs} * T$$

where T is the thickness of the bin (m).

Monthly 24-hour gill netting events were completed on each lake to obtain species composition information. We used stretch mesh sizes of 19, 25, 32, 51, 64, and 89 mm.

*Zooplankton Biomass Hydroacoustic Regression*

We developed a regression to convert 430kHz acoustic data to zooplankton biomass estimates. We incorporated data from three separate lakes in order to build a strong and more predictive relationship between zooplankton biomass and acoustic information. We sampled Crystal Lake and Sparkling Lake, which is very similar to Crystal Lake but is slightly more productive. Sparkling Lake is an oligotrophic seepage lake, and is also an NTL-LTER research lake. We also incorporated previously gathered zooplankton biomass/acoustic measurements from Lake Superior (Holbrook 2011).

These data were obtained using the same methods as in our study. Combining these three lakes which span a range of oligotrophic lake conditions and their associated zooplankton communities facilitated the development of a more robust backscatter to biomass relationship.

Zooplankton were collected at ten pelagic locations on Crystal (Figure 1) and Sparkling (Figure 2) Lakes during one night sampling event (July 9, 2014). A 0.5 diameter 153um mesh size Wisconsin net was towed from 1.5m above the lake bottom to the surface. Two replicates were taken with each net. Samples were preserved in glass jars in 90% ethanol. In the laboratory, zooplankton were sorted to species, except *Diaptomus spp.*, and cyclopoid and calanoid copepids. Hensen-Stempel pipettes were used to subsample three 1 ml aliquots, and we ensured at least 100 individuals were counted. All individuals were identified and counted in each aliquot. Five individuals in each species/taxon were measured for length. *Cladocera* were measured from the top of the head to the base of the caudal spine and *Copepoda* were measured from the top of the head to the insertion of spines on the caudal ramus (GLNPO 2003).

To convert Wisconsin net data to biomass, a basic length-mass model was used:

$$\ln(w) = \ln(a) + b\ln(L)$$

where ln *(w)* is the natural logarithm of estimated dry weight (µg), *a* and *b* are species-specific constants, and ln (*L)* is the mean of natural log transformed lengths measurements (mm) of species within each sample (GLNPO 2003). Acoustic sampling with the 430 kHz transducer was completed at the exact location of the ten different pelagic sample locations on each lake.

We then developed a regression to convert acoustic backscatter to biomass. We used the Area Backscatter Coefficient (ABC) from acoustic data, which is the strength of backscattering per meter:

$$ABC = 10^{\frac{S_v}{10}}T \ [\mathrm{m}^2/\mathrm{m}^2]$$

where $S_v$ is the mean volume backscattering strength of the domain being integrated, and $T$ is the thickness of the domain being integrated (average depth within each horizontal bin).

Fish targets were eliminated manually in Echoview during post-processing prior to formal analysis. ABC was analyzed down to 1.5 meters from the bottom of the lake in the 20logR domain for echo integration. ABC was regressed against zooplankton dry weight biomass estimates (mg) from the Wisconsin nets in order to establish a relationship between the two variables. We used the regression to estimate zooplankton biomass for each sampling date, and did not apply the 95% confidence intervals to the estimates.

*Semivariogram analysis*

Although acoustic sampling was most often completed every other week, some survey dates were lacking GPS coordinates due to intermittent equipment failure. Other data sets showed signs of interference by large insect hatches. In order to ensure the most accurate results, we chose to omit these sample dates in order to use only the most accurate and robust data for analysis.

We developed semivariograms for each sampling date for fish and zooplankton. Semivariograms represent the sums of squared differences for all points separated by a selected distance, called the lag distance (Durance et al. 2006):

$$\gamma(h) = \frac{1}{2n(h)} \sum_{i} [Z(x_i - Z(x_i + h)]^2$$

where *n* is the number of pairs of sample points separated by distance *h*, and $Z(x_i)$ is the density (fish) or biomass (zooplankton) estimate at point $x_i$. All geostatistical data are spatially autocorrelated, where objects that are located close together are more similar than objects farther apart from each other. Semivariograms indicate how autocorrelation decreases with increasing distance (ESRI 2015). Semivariogram models depict semivariance increases until a certain distance, and then stabilizes. The distance at which the semivariance stabilizes is called the range, which indicates the distance where samples are no longer autocorrelated and become independent. Distances below the range are autocorrelated and are not spatially independent. Range values also give insight regarding size of areas with similar densities (Durance et al. 2006).

We used spherical semivariogram models because they provided the most accurate predictions; in almost every sampling date, spherical modeling yielded the root mean square standardized prediction error closest to one (indicating predictions are close to measured values) and mean standardized prediction error closest to zero (indicating predictions are unbiased) (ESRI 2015).

In choosing a horizontal bin size for analysis, there is a tradeoff between fine scale resolution and minimizing bins with zero fish that result in zero density estimates. Further, Parker-Stetter et al. (2009) recommend using bin sizes less than half of the range

value estimates from semivariogram analysis. We tested bin sizes of 25, 50, and 75m and found that 25m often resulted in empty bins, and chose to use the smallest bin size with a sufficient number of targets for density estimates (50m). Although 50m is greater than half the range for some sample dates (Table 2, Table 3), we chose to use 50m bin sizes to decrease zero density estimates. Use of 50m bin sizes also resulted in low to no presence of the nugget effect. Theoretically, at separation distances of zero, there should be zero semivariance. However, semivariograms often have a value greater than zero for semivariance at distance zero. Values greater than zero indicate the "nugget effect," suggesting there is spatial variability at scales smaller than those used for analysis (ESRI 2015). Therefore, low nugget values indicate adequate sample resolution while higher nugget values would necessitate exploring finer spatial scales (Durance et al. 2006).

We determined autocorrelation between fish and zooplankton aggregations for each sampling date by calculating cross variograms:

$$\gamma_{AB}(h) = \frac{1}{2n(h)} \sum_i^n \sum_j^m \{Z_a(x_i) - Z_a(x_j)\}\{Z_b(x_i) - Z_b(x_j)\}$$

where $n$ is the number of pairs of observations separated by distance $h$ for the first variable, $Z_a$, and the second variable, $Z_b$, at points $x_i$ and $x_j$.

We tested for effects of season and mixing on range values from semivariogram and cross variogram analysis. We defined spring as any date on or prior to June 30 (fish N=4, zooplankton N=5), summer as dates ranging July1-August 31 (fish N=12, zooplankton N=12), and fall as September 1 and later (fish N=7, zooplankton N=5).

We used density estimates of fish and biomass estimates of zooplankton to examine spatial correlations for each sampling date by plotting zooplankton density against fish density for each 50m bin within the sample date. We analyzed the relationship for significance and whether it was positive or negative using linear regression.

We log-transformed fish and zooplankton data to normalize density data, and also because it may be easier to detect spatial autocorrelation with log-transformed data (Rivoirard et al. 2008). We used Kruskal-Wallis tests to analyze for significant effects of season and mixing on semivariogram estimates because Kruskal-Wallis tests account for any nonparametric data and different sample sizes.

## Results

### *Lake Mixing Results*

Lawson et al. (2015) determined Crystal Lake was fully destratified during manipulation years (2012 and 2013). In pre and post manipulation years, stratification set up in the spring and persisted through the summer until fall turnover. During manipulation, the hypolimnion increased in temperature until the entire water column was isothermic by August and remained isothermic until ice formation (Lawson et al. 2015). Maximum lake temperatures during manipulation exceeded most critical temperatures for smelt, including preferred (12$^{o}$C), upper avoidance (15$^{o}$C), and lethal temperatures (22$^{o}$C) (Furgeson1965, Wismer and Christie 1987, Lantry and Stewart 1993, Lawson et al. 2015).

*Zooplankton Biomass Hydroacoustic Regression*

The Crystal Lake, Sparkling Lake, and Lake Superior Wisconsin net samples contained variable proportions of taxonomic groups (Figure 3, see Holbrook 2011 for Lake Superior data). Crystal Lake consisted mainly of Cyclopoid copepods, Daphnids, and other Cladocera with a small proportion of Calanoid copepods. Sparkling Lake consisted mainly of Cyclopoid copepods and Calanoid copepods. Lake Superior largely consisted of Calanoid copepods with some Cyclopoid copepods and Daphnids.

We found a significant linear relationship ($p < 0.0001$) between logged zooplankton biomass estimates from Wisconsin nets ($mg/m^2$) and ABC ($m^2/m^2$) despite differences in species compositions across lakes (Figure 4). The relationship was (N=36, $F=118.1$, $R^2= 0.83$, $p<0.0001$):

$$\log\left[Zooplankton\ Biomass\ \left({}^{mg}/_{m^2}\right)\right] = 0.73 * \log\left[ABC\ \left({}^{m^2}/_{m^2}\right)\right] + 8.04$$

The regression equation was applied to the acoustic data to estimate zooplankton biomass.

*Zooplankton Spatial Analysis*

Mixing did not have a significant effect on average zooplankton biomass (Table 1, Kruskal-Wallis, H=2.8, df=2, p=0.24), but there was a significant seasonal effect on biomass (Kruskal-Wallis, H=11.3, df=2, p=0.003). Range estimates (m) for zooplankton populations were not significantly affected by mixing (Kruskal-Wallis, H=0.14, df=2, p=0.93). However, we found significant seasonal effects on zooplankton spatial structure (Figure 5**,** Kruskal-Wallis, H=7.5, df=2, p=0.02). The range estimates for zooplankton in spring and summer were 130±56m (95% CI) and 127±57m (95% CI), respectively. This

distance increased in the fall to 282±87m (95% CI). We regressed zooplankton biomass estimates against range values to determine if low zooplankton densities in the fall were causing variability around average range values, and did not find an effect of biomass estimates on the range values (N=18, F=2.25, p=0.15, $R^2$=0.1).

*Fish Spatial Analysis*

Contrary to zooplankton, we did not find a significant seasonal effect on the range estimates for fish (Kruskal-Wallis H=2.3, df=2, p=0.31), or seasonal effect on biomass estimates (Table 1, Kruskal-Wallis H=5.7, df=2, p=0.58), but did find significant effects of lake mixing on range values (Figure 6, Kruskal-Wallis, H=9.8, df=2, p=0.0075). During pre-manipulation years fish indicated autocorrelation at distances less than 171±52m (95% CI), which significantly decreased to an average of 68±32m (95% CI) during mixing. Post manipulation range estimates increased to 192±62m (95% CI).

*Interactions between Fish and Zooplankton*

Cross-variograms showed significant spatial autocorrelation between fish and zooplankton at range values 80-315m and below (Table 3, Wilcoxon Rank Test, W=1, p=0.0004). We did not detect range values below 80m for dates where we detected significant cross variograms. We characterized significant autocorrelation by cross variograms fitting the standard semivariogram curve where semivariance increases with distance and then levels off (Figure 7), and non-significant autocorrelation as models as variograms with points without pattern (Figure 8). There was no significant relationship between range values and mixing (Kruskal-Wallis, H=1.3, df=2, p=0.52) or season

(Kruskal-Wallis, H=0.49, df=2, p=0.78). For autocorrelated cross-variograms, our linear regressions indicated a positive relationship between fish and zooplankton. We did not detect negative relationships between fish and zooplankton in any of our sample dates.

## Discussion

### *Zooplankton spatial distributions*

We identified a biomass/ABC regression using backscatter from 430kHz acoustic data that was statistically robust with good predictive power. Although Crystal Lake, Sparkling Lake, and Lake Superior have different zooplankton species compositions, these differences did not cause deviations in our regression, suggesting the relationship is durable and broadly representative of variable species compositions in oligotrophic lakes. Our regression provides a means to quickly measure zooplankton biomass over a large spatial scale at a fine resolution in oligotrophic lakes. Acoustic estimates of zooplankton biomass are less time intensive than traditional methods of zooplankton sampling, such as Wisconsin nets, and are less prone to error of naturally patchy distributions of zooplankton, as nets may be.

With mixing, we drastically manipulated internal biological and physical components of Crystal Lake previously found to affect zooplankton distributions, but seasonal patterns in horizontal distributions persisted (Hanski 1981, Folt and Burns 1999, Ware and Thomson 2005). Manipulation resulted in elimination of stratification, and altered zooplanktivorous fish behavior (Figure 6). Any prey behavioral movements in response to change in predator behavior are more likely to be observed on scales smaller than those used in our analysis (~1m) (Folt and Burns 1999, Thackeray et al. 2004). Further,

zooplankton are weak swimmers, and so any movement they undergo in response to predatory avoidance is likely to be overridden by large scale physical processes (Thackeray et al. 2004, Blukacz et al. 2009). We also observed changes in zooplankton species composition with mixing. In 2012 *Leptodora kindtii*, a predatory cladoceran, greatly increased in abundance, and *Daphnia mendotae*, a grazing cladoceran, decreased, likely due to increased predatory pressure from *Leptodora kindtii* (Lawson unpublished data, 2013).

Persistent seasonal trends despite changes within the ecosystem suggest horizontal patterns of zooplankton distributions are driven by external forces, such as wind and wind-induced water movements, which we did not alter with our manipulation (George and Winfield 2000, Blukacz et al. 2009, Rink et al. 2009). Because large scale spatial patterns are often the result of large scale processes, our consistently high range estimates throughout all seasons and lack of change with mixing support previous research findings that large scale abiotic processes such as wind-induced water movements are major drivers of horizontal zooplankton distributions (George and Winfield 2000, Thackeray 2004, Blukacz et al. 2009).

It is possible that some fall sample dates occurred after prolonged wind events, causing zooplankton redistribution and higher range values (George and Winfield 2000, Blukacz et al. 2009). We do not expect seasonal variability to be caused by spring and fall turnover because we did not observe similar trends in the spring. Furthermore, we do not associate seasonal patterns with seasonal changes in sunlight intensity. If this were the case we would expect spring and fall to show similar patterns in spatial distributions.

However, it may be difficult to detect spring and fall effects on horizontal distributions because we only had five spring sampling dates.

*Fish spatial distributions*

Our results suggest that thermal destratification of Crystal Lake decreased range values for fish, and that fish returned to pre-manipulation distributions after mixing ceased. That is, the shift in aggregation size was a plastic behavior in response to mixing-induced changes in thermal habitat. Our calculated change in aggregation behavior agrees with observed changes in behavior by Lawson et al. (2015) during the manipulation. Lawson et al. (2015) detected novel rainbow smelt behavior when rainbow smelt were observed near shore and at the surface of the lake during the day. We hypothesize this shift is likely due to a behavioral response to increase in lake temperatures beyond rainbow smelt thermal optima with mixing, and elimination of preferred cold water habitat. Change in behavior may indicate searching behavior for more suitable conditions. This stress response hypothesis is further supported by an observed decrease in smelt body condition during the manipulation, which indicates a decline in population performance (Neumann and Willis 1996, Lawson et al. 2015).

Our results are consistent with several studies on the effects of increasing water temperature in which cold water fish abandon normal behavior and employ behavioral mechanisms in response to increased production of anaerobic byproducts (Breau et al. 2011). Breau et al. (2011) found that at high temperatures, fish attempt to decrease physiological imbalances when unable to support increases in basal metabolism. These behaviors are often seen as “flight,” or searching responses, where interspecific

interactions, such as schooling, decrease (Robinson and Pitcher 1989, Breau et al. 2011). It is possible that our manipulation induced a flight response in rainbow smelt, where fish began to swim separately in smaller aggregations in order to reduce intraspecific competition for resources required for increased metabolism, and to seek out cooler water (Berman and Quinn 1991, Robinson and Pitcher 1989, Breau 2011).

Changes may also have been due to redistribution of other required resources, such as prey, or a change in water clarity. During manipulation years we observed a decrease in Secchi depth compared to historical LTER data. Decreased light availability may decrease reaction distance and therefore capture ability for prey, requiring smelt to adapt new, more dispersed, behaviors to increase consumption (Aksnes and Giske 1993, Vogel and Beauchamp 1999). However, we would expect change in water clarity to have a greater effect on day distributions because fish are already light-limited at night (Vogel and Beauchamp 1999). It is also likely that change in water clarity could affect fish distributions at a vertical scale, as light is a major driver of DVM behavior (Mehner et al. 2012). We do not believe the changes in fish aggregations were a result of redistribution in prey, because we did not observe a change in zooplankton distributions during manipulation years. It is possible fish distribution changed due to redistribution of chlorophyll or dissolved oxygen (Rinke et al. 2009), however, we do not have spatial measures or whole-lake distributions of those variables.

*Spatial relationships and interactions between fish and zooplankton*

Our cross variogram results indicate fish and zooplankton are autocorrelated, on average, at distances less than 188m. All but one sample date indicate ranges 156m-

315m. During autocorrelated sample dates, fish and zooplankton were positively correlated, suggesting fish were aggregating in areas of high zooplankton density. We did not detect negative relationships. Our results suggest an absence of top-down effects of fish on zooplankton aggregations in our study system, or that these interactions are occurring at scales we did not measure.

Because there was no relationship between spatial covariation of fish and zooplankton with season or lake mixing, we hypothesize that zooplankton are bottom-up drivers of fish distributions for at least part of the year. For sample dates where we did not detect covariation, if there are multiple interacting drivers of fish distributions, it is possible that we did not detect positive relationships due to interactions between other variables, or that interactions between fish and zooplankton are occurring at scales other than what we measured. Our results are consistent with Lezama et al. (2006) who detected inter-annual variability in cross variogram results for fish and zooplankton.

Lack of correlation on some dates may indicate dietary shifts in fish. Rainbow smelt, which make up over 99% of pelagic fish in Crystal Lake, have diverse diets that vary seasonally (Evans and Loftus 1987, Isaac et al. 2012). Although we did not detect seasonal effects on correlations, it is possible fish were selecting for different prey during some of the sample dates (e.g. *Chironomid* larvae). Further, we do not expect interspecific top-down effects, where large smelt prey on smaller smelt, to be masked by our analysis due to lack of piscivory by the smelt in Crystal Lake (Dobosenki, unpublished data). Examinations of prey selection are ongoing and may shed light on

changes in feeding on zooplankton and potential influences on spatial covariation between zooplankton and fish.

We observed little evidence of top-down effects of planktivory on zooplankton aggregations. Our results indicate fish are concentrating in areas of higher zooplankton biomass during portions of the year. If prey are mobile, they may attempt to avoid predation, resulting in a negative correlation between plankton and their predators (Krebs 1978, Rose and Leggett 1990**)**. However, if prey are relatively immobile and do not have access to refuge areas, positive spatial correlation is likely to occur (Sih 1984). In oligotrophic systems such as Crystal Lake, zooplankton are likely to remain in high-risk regions near the surface in order to maximize consumption because they are food limited (Lampert 1989, Dodson 1990). Further, rainbow smelt in Crystal Lake occupy the hypolimnion, and so it is likely zooplankton maintain similar vertical positions from day to night to decrease spatial overlap with predators (Young and Yan 2008).

We used a general measure of zooplankton biomass that may not be solely representative of the zooplankton fish are selecting for in this system. Zooplanktivorous fish are primarily visual predators, and therefore select the largest zooplankton that are most visible (Brooks and Dodson 1965, Lathrop et al 2002). Our 430kHz acoustic/biomass regression measured total zooplankton biomass due to difficulties in sorting out specific taxonomic groups in acoustic data. Our results were most consistent with those of Rinke et al. (2009) who found a bottom-up relationship between overall zooplankton biomass and fish density. In order to detect species-specific interactions, our regression would need to be used in conjunction with other sampling techniques such as

net tows or optical plankton counters. Future analyses that explore spatial patterning in species-specific zooplankton-fish interactions may detect top-down planktivory effects at small spatial scales.

During sample dates where spatial covariation was not detected, fish and zooplankton may be interacting at scales smaller than our measurements. While we used the smallest spatial resolution reasonable for the detection of adequate backscatter from both fish and zooplankton, examining more productive lakes may allow researchers to decrease segment size below 50m, which we chose in order to reduce error in semivariogram analysis. Another option less prone to error would be to increase total transect length to provide additional sampling points that are closer together in order to increase paired data at shorter distances. Measuring at finer scales may also provide an opportunity to examine negative depletion relationships, where fish are initially drawn to areas of high zooplankton biomass (positive relationships), then deplete zooplankton biomass through predation (Lezama et al. 2011).

Our research has identified several new questions that require additional research. We did not test for species- or size-specific interactions of fish and zooplankton, which have been observed in several other studies (Brooks and Dodson 1965, George and Winfield 2000, Durance et al., 2006). Additionally, daytime spatial relationships may exhibit different patterns than observed at night. Daytime analysis may yield insight on potential drivers of fish schooling, because zooplanktivorous fish disperse at night. However, daytime fish acoustic data may be prone to high variability due to intermittent areas of high and low fish densities (Appenzeller and Leggett 1992). There may also be a

difference in spatial relationships in vertical distributions of fish and zooplankton. Lawson et al. (2015) observed change in rainbow smelt vertical distributions when authors noted daytime surfacing of smelt during the manipulation. Further, zooplankton may have increased vertical dispersion in response to warming of deeper waters and mixing-induced algae blooms. Our horizontal analysis may not have detected these changes in vertical distributions.

To our knowledge, this is the first case study that has concurrently examined horizontal spatial relationships of fish and zooplankton at a relatively fine resolution in a small lake. Further, we tested durability of these patterns in response to whole-lake destratification. We found that fish may respond horizontally to disturbance of vertically structured processes, especially when fish are reliant on depth-dependent factors such as cold water. Our results also agree with the hypothesis that large-scale abiotic events such as wind-induced water movements are seemingly the strongest drivers of zooplankton horizontal distributions. Because patterns of fish and zooplankton distributions occur at different spatial scales and result from different processes, it is important to measure each group at the appropriate scale. Factors affecting fish distributions at one scale may be vastly different than factors affecting zooplankton at the same scale. It is possible that scale-dependent relationships may be system-dependent and vary based on physical and biological characteristics of the particular system (Durance et al. 2006). Further, ecological conclusions from one scale within the same system may be different from conclusions based off sampling at a different scale (Durance et al. 2006). Geostatistics, such as the semivariogram analysis used in this study, provide a means to guide

hypothesis testing and subsequent management decisions by indicating distances at which samples are spatially independent and determining relative importance of variables across scales.

Table 1. Average fish densities and zooplankton biomasses and associated standard deviations for each sample date, estimated via hydroacoustic surveys.

| Sample Date | Average Fish Density (#/ha) | Fish Standard Deviation | Average Zooplankton Biomass ($mg/m^2$) | Zooplankton Standard Deviation |
|---|---|---|---|---|
| June 4 2010 | 4624 | 1656 | 4706 | 546 |
| June 30 2010 | - | - | 4165 | 394 |
| July 13 2010 | 6919 | 5848 | 2152 | 504 |
| Oct. 31 2010 | 1461 | 2755 | 875 | 317 |
| July 12 2011 | - | - | 3248 | 383 |
| July 28 2011 | 5639 | 2406 | 3143 | 253 |
| Aug. 15 2011 | 8446 | 3180 | - | - |
| May 31 2012 | 1375 | 995 | 3181 | 339 |
| June 30 2012 | 4039 | 4472 | 4415 | 258 |
| July 19 2012 | 4565 | 3157 | 5952 | 1890 |
| Aug. 06 2012 | 1987 | 1758 | 3928 | 1276 |
| Aug. 22 2012 | 2544 | 1600 | - | - |
| Sept. 13 2012 | 1413 | 1231 | - | - |
| Sept. 28 2012 | 487 | 681 | - | - |
| Oct. 11 2012 | 1493 | 2702 | 867 | 280 |
| Nov. 08 2012 | 2907 | 3569 | 524 | 214 |
| July 02 2013 | 1401 | 1916 | 1704 | 222 |
| July 16 2013 | 3480 | 2754 | 6103 | 1967 |
| July 29 2013 | - | - | 3853 | 1710 |
| Aug. 15 2013 | 1465 | 818 | 5810 | 2990 |
| June 17 2014 | 1630 | 1067 | 4060 | 364 |
| July 09 2014 | 1605 | 1016 | 3985 | 319 |
| July 31 2014 | 2297 | 2186 | 1286 | 220 |
| Aug. 06 2014 | 2588 | 1765 | 1636 | 307 |
| Sept. 20 2014 | 1769 | 1939 | 809 | 382 |
| Oct. 11 2014 | 967 | 1000 | 991 | 391 |

Table 2. Semivariogram results for log fish density and log zooplankton biomass. All semivariogram analysis was conducted using spherical models and a 50m segment size.

| Sample Date | Log Fish Density | | | | Log Zooplankton Density | | | |
|---|---|---|---|---|---|---|---|---|
| | Maximum Distance Between Points | Nugget | Sill | Range (m) | Maximum Distance Between Points | Nugget | Sill | Range (m) |
| June 4 2010 | 488.06 | 0.00 | 0.03 | 66.69 | 488.06 | 0.00 | 0.00 | 174.24 |
| June 30 2010 | - | - | - | - | 488.15 | 0.00 | 0.00 | 183.95 |
| July 13 2010 | 545.33 | 0.05 | 0.05 | 202.30 | 545.25 | 0.01 | 0.00 | 56.82 |
| Oct. 31 2010 | 373.98 | 0.06 | 0.02 | 191.48 | 373.90 | 0.00 | 0.01 | 163.28 |
| July 12 2011 | - | - | - | - | 534.08 | 0.00 | 0.00 | 189.73 |
| July 28 2011 | 532.72 | 0.01 | 0.02 | 196.72 | 532.56 | 0.00 | 0.00 | 22.00 |
| Aug. 15 2011 | 529.45 | 0.00 | 0.05 | 197.41 | - | - | - | - |
| May 31 2012 | 531.50 | 0.13 | 0.07 | 38.09 | 531.78 | 0.00 | 0.00 | 111.67 |
| June 30 2012 | 488.06 | 0.06 | 0.00 | 78.57 | 488.15 | 0.00 | 0.00 | 146.59 |
| July 19 2012 | 515.53 | 0.02 | 0.01 | 90.21 | 523.31 | 0.00 | 0.00 | 103.50 |
| Aug. 06 2012 | 518.09 | 0.19 | 0.10 | 62.80 | 514.22 | 0.01 | 0.01 | 105.28 |
| Aug. 22 2012 | 480.26 | 0.00 | 0.27 | 30.66 | - | - | - | - |
| Sept. 13 2012 | 516.68 | 0.00 | 0.33 | 27.53 | - | - | - | - |
| Sept. 28 2012 | 550.94 | 0.15 | 0.05 | 14.19 | - | - | - | - |
| Oct. 11 2012 | 495.65 | 0.87 | 0.01 | 11.01 | 524.93 | 0.00 | 0.02 | 200.72 |
| Nov. 08 2012 | 520.70 | 0.00 | 0.71 | 26.03 | 520.90 | 0.00 | 0.02 | 289.59 |
| July 02 2013 | 556.63 | 0.06 | 0.10 | 96.13 | 535.81 | 0.00 | 0.00 | 116.70 |
| July 16 2013 | 520.67 | 0.00 | 0.15 | 208.95 | 520.34 | 0.00 | 0.01 | 314.51 |
| July 29 2013 | - | - | - | - | 555.18 | 0.00 | 0.04 | 128.77 |
| Aug. 15 2013 | 515.49 | 0.00 | 0.05 | 74.80 | 515.48 | 0.00 | 0.04 | 144.24 |
| June 17 2014 | 530.28 | 0.03 | 0.03 | 275.65 | 530.12 | 0.00 | 0.00 | 32.60 |
| July 09 2014 | 530.28 | 0.03 | 0.03 | 301.87 | 530.12 | 0.00 | 0.00 | 32.06 |
| July 31 2014 | 489.62 | 0.27 | 0.02 | 136.35 | 489.57 | 0.00 | 0.01 | 278.45 |
| Aug. 06 2014 | 517.88 | 0.10 | 0.04 | 112.58 | 517.87 | 0.01 | 0.00 | 28.27 |
| Sept. 20 2014 | 622.80 | 0.17 | 0.34 | 175.01 | 623.02 | 0.00 | 0.08 | 391.30 |
| Oct. 11 2014 | 522.07 | 0.14 | 0.89 | 152.70 | 522.01 | 0.00 | 0.04 | 364.82 |

Table 3. Cross variogram results for fish and zooplankton. All cross variogram analysis was conducted using spherical models and a 50m segment size. An "X" under "Autocorrelation" indicates a significant relationship.

| Sample Date | Nugget | Sill | Range (m) | Autocorrelation |
|---|---|---|---|---|
| June 04 2010 | 0.013 | 0.016 | 51.33 | - |
| July 13 2010 | 0.037 | 0.0372 | 31.8 | - |
| Oct. 31 2010 | 0.023 | 0.043 | 156.8 | X |
| July 28 2011 | 0.01 | 0.018 | 315.7 | X |
| May 31 2012 | 0.07 | 0.11 | 31 | - |
| June 30 2012 | 0.0017 | 0.0417 | 206 | X |
| Aug. 06 2012 | 0.04 | 0.06 | 178 | X |
| Oct. 11 2012 | 0.3 | 0.45 | 30.1 | - |
| Nov. 08 2012 | 0.3 | 0.36 | 30 | - |
| July 02 2013 | 0.04 | 0.067 | 35.11 | - |
| July 16 2013 | 0.03 | 0.057 | 31.8 | - |
| Aug. 15 2013 | 0.06 | 0.04 | 142 | - |
| June 17 2014 | 0.03 | 0.029 | 32.3 | - |
| July 09 2014 | 0.03 | 0.029 | 32.3 | - |
| July 31 2014 | -0.047 | 0.083 | 47.4 | - |
| Aug. 06 2014 | 0.04 | 0.0655 | 29.5 | - |
| Sept. 20 2014 | 0.15 | 0.25 | 81.6 | X |
| Oct. 11 2014 | 0.098 | 0.131 | 25 | - |

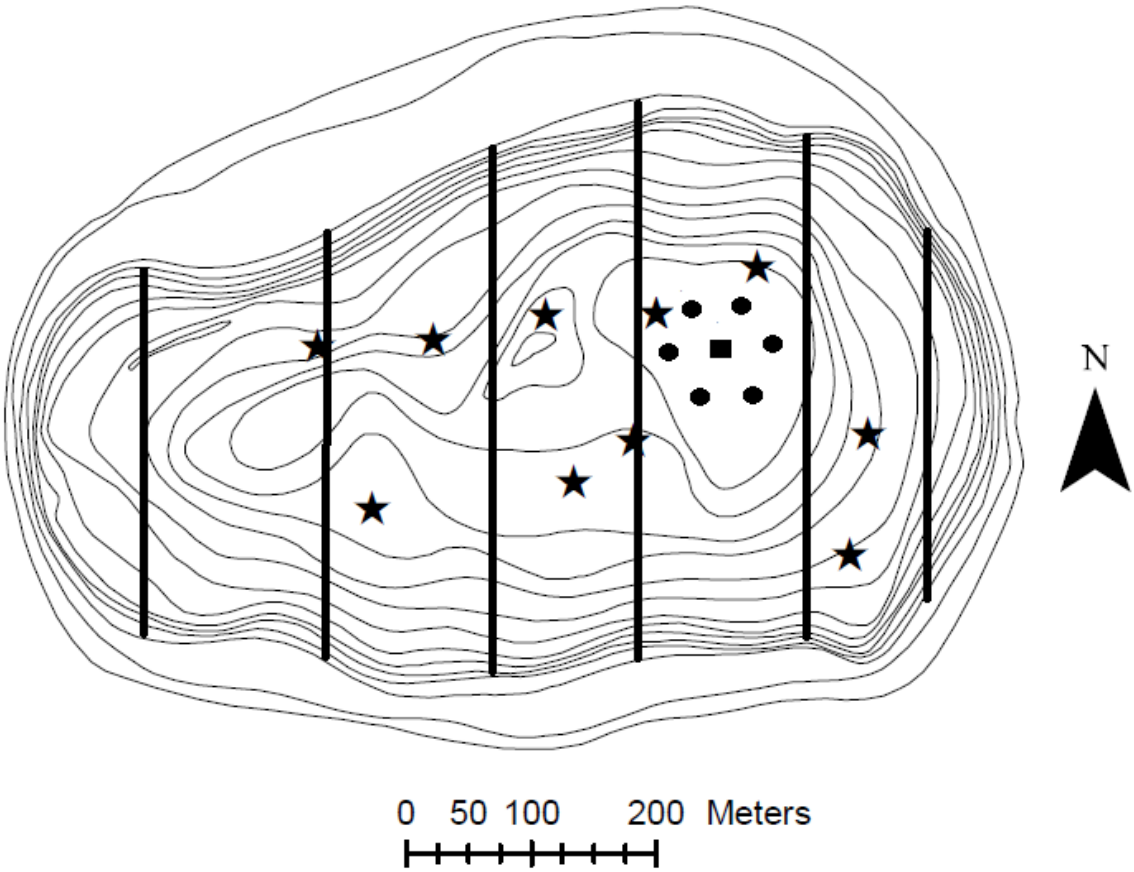


Figure 1. Hydroacoustic survey transects on Crystal Lake for 2013 and 2014 (vertical black lines), location of Wisconsin net and hydroacoustic zooplankton sampling for linear regression (★), location of GELIs (●), and location of GELI control center (■). Depth contours are 5 foot intervals (Lake District Bathymetry, NTL-LTER).

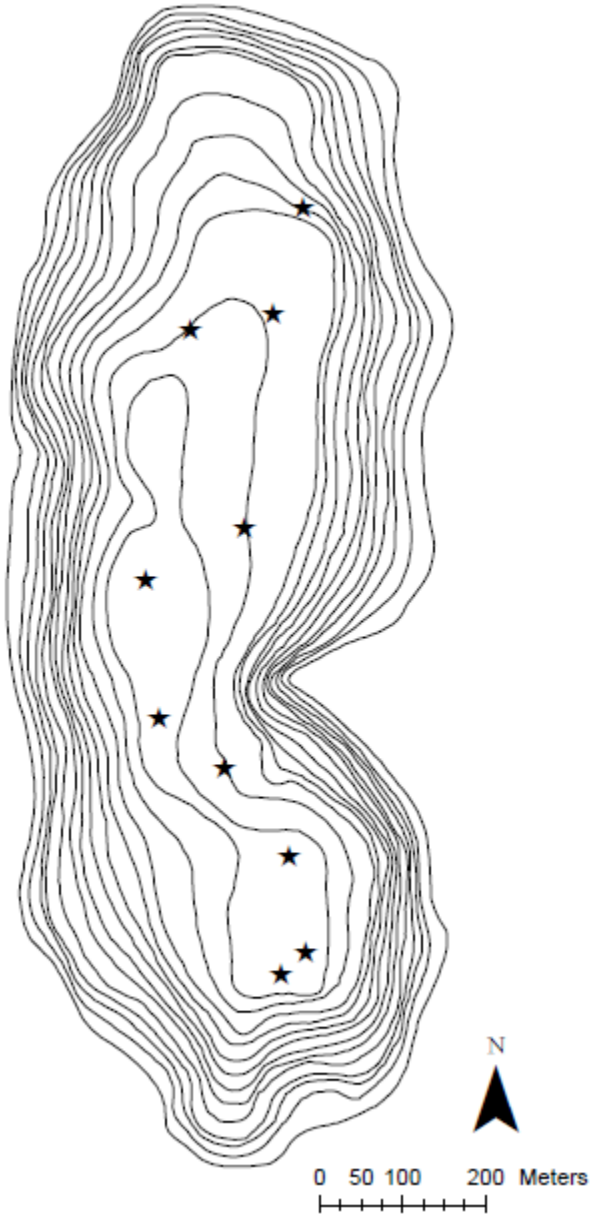


Figure 2. Locations of Wisconsin net and hydroacoustic zooplankton sampling for linear regression on Sparkling Lake (★). Depth contours are 5 foot intervals (Lake District Bathymetry, NTL-LTER).

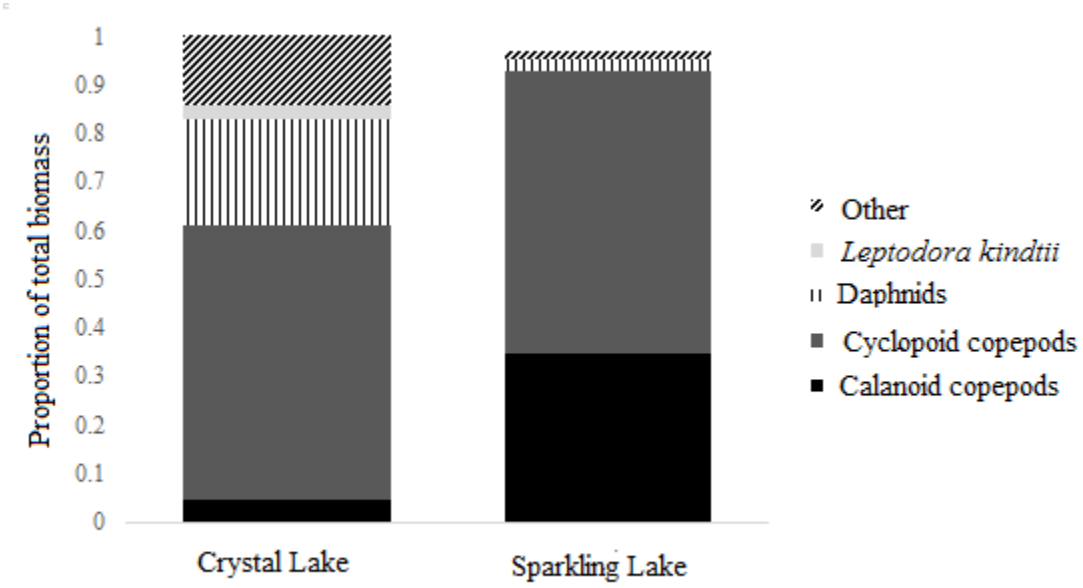


Figure 3. Relative proportion of dry weight (mg) biomass for zooplankton taxonomic groups from Crystal and Sparkling Lakes used to develop regression between 430 kHz hydroacoustic data and zooplankton biomass estimates from Wisconsin tows.

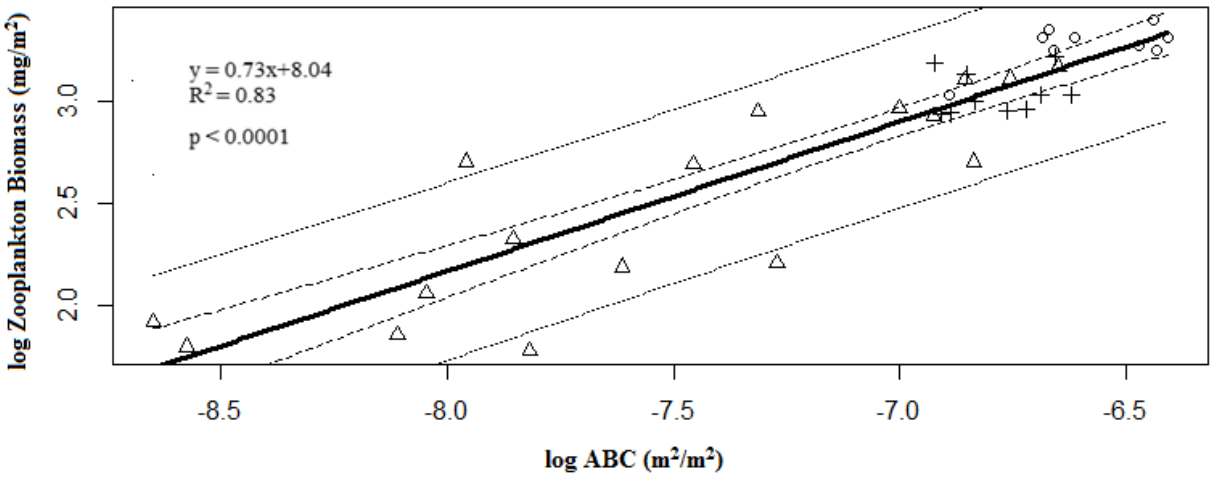


Figure 4. Linear relationship between site-specific log zooplankton biomass estimates ($mg/m^2$) from Wisconsin net tows and 430kHz log hydroacoustic backscatter ($m^2/m^2$) (N=36). The innermost solid line represents the ordinary least squared regression trend line, inner dashed lines represent the 95% confidence intervals, and the outer dotted lines represent 95% prediction intervals. (+) indicates Sparkling Lake, (o) indicates Crystal Lake, and (Δ) indicates Lake Superior.

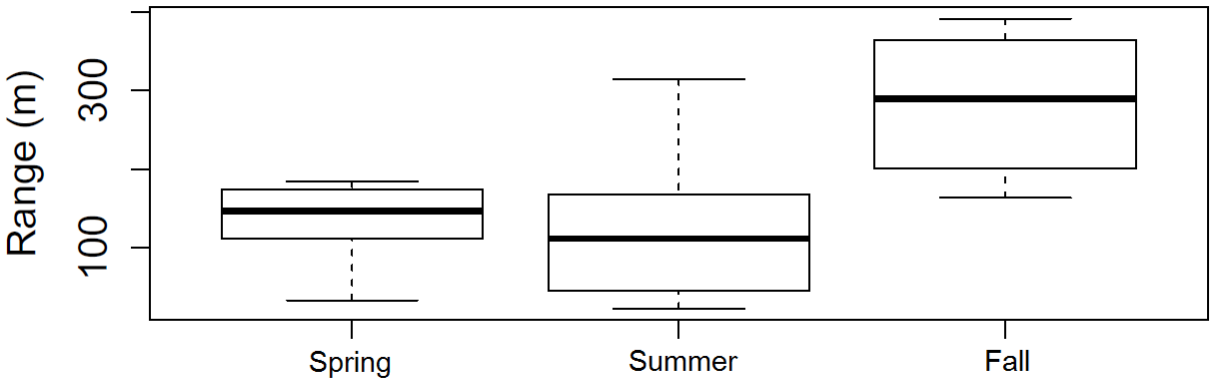


Figure 5. Box plots for range values (m) from zooplankton semivariograms for each season (Spring: 130±56m N=5, Summer: 127±57m N=12, Fall: 282±87m N=5) 2010-2014. Dark bars indicate median range values, top and bottom of boxes indicate 25$^{th}$ and 75$^{th}$ quartiles, and lower and upper capped bars indicate minimum and maximum calculated range values.

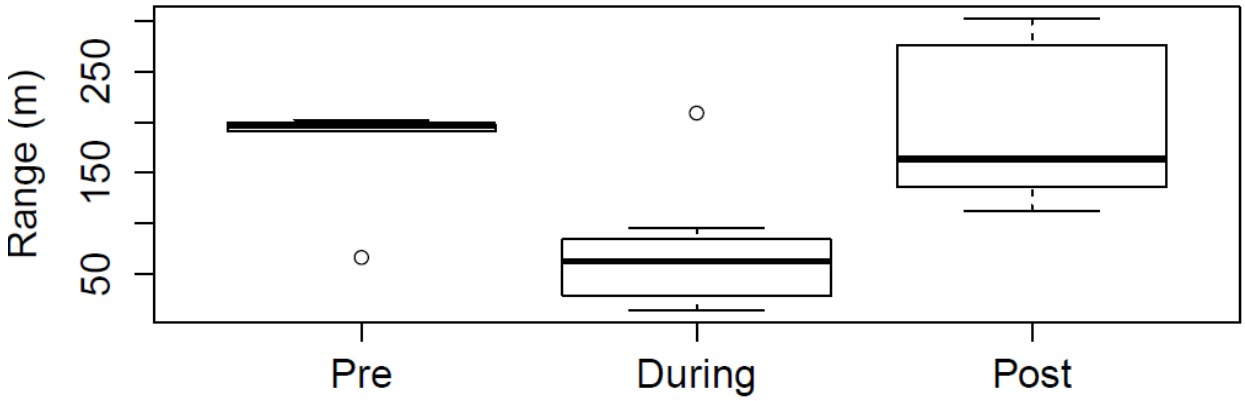


Figure 6. Box plots for range values (m) from fish semivariograms for "Pre" lake mixing (2010 and 2011, N=7), "During" lake mixing (2012 and 2013, N=12), and "Post" lake mixing (2014, N=6). Dark bars indicate median range values, top and bottom of boxes indicate 25$^{th}$ and 75$^{th}$ quartiles, lower and upper capped bars indicate minimum and maximum calculated range values, and hollow circles indicate outliers.

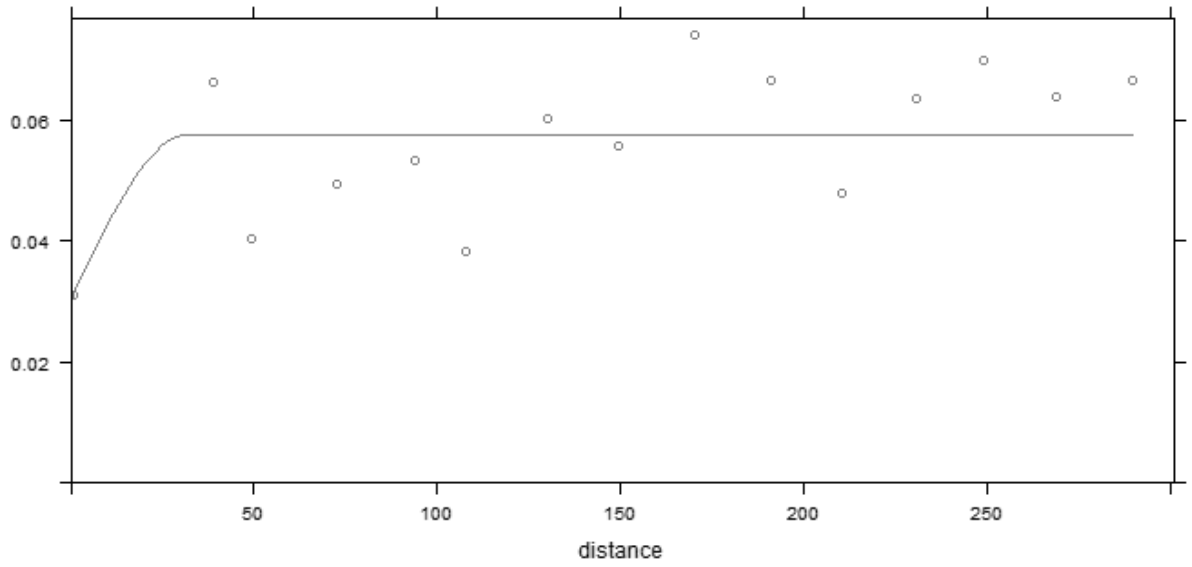


Figure 7. Example from July 16 2012 of a cross variogram for fish and zooplankton indicating no autocorrelation.

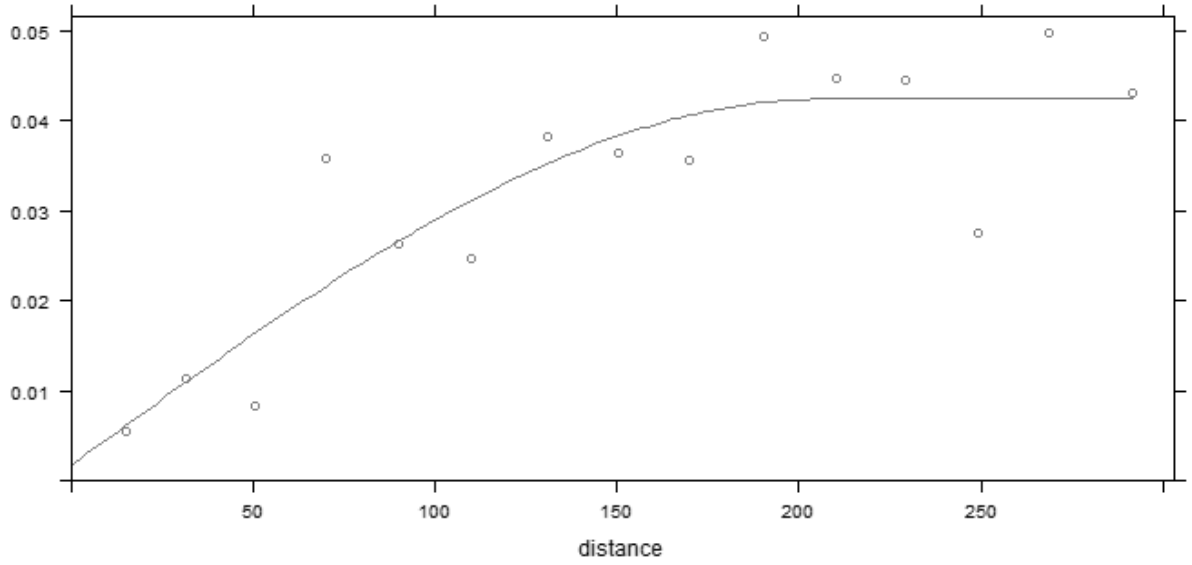


Figure 8. Example from Oct. 31 2010 of a cross variogram for fish and zooplankton indicating spatial autocorrelation at range value 156m.

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